Metabolomic characterization of renal cell carcinoma using high-resolution magic angle spinning magnetic resonance spectroscopy

Melissa J. Huynh[1] MD, Andrew Gusev[1], Francesco Palmas[3] PhD, Lindsey Vandergrift[3] BA, Chin-Lee Wu[2] MD Leo Cheng[2,3] PhD, Adam S. Feldman[1] MD, MPH

[1]Department of Urology, Massachusetts General Hospital, Boston, MA, USA
[2]Department of Pathology, Massachusetts General Hospital, Boston , MA, USA
[3]Department of Radiology, Massachusetts General Hospital, Boston , MA, USA
[4]Harvard Medical School, Boston , MA, USA

INTRODUCTION

- Renal cell carcinoma (RCC) is known to be a metabolic disease, with the various RCC subtypes exhibiting aberrations in several different metabolic pathways.
- Metabolomics measures global metabolite profiles from various metabolic pathways, as these profiles are influenced across a pathological progression
- ***We investigated the metabolomic profile of renal cell carcinoma and compared it to that of adjacent benign renal parenchyma using high resolution magic angle spinning (HRMAS) magnetic resonance spectroscopy (MRS).***

METHODS

- **Samples:** 38 RCC samples from partial or radical nephrectomy were stored in frozen tissue bank
  - 16 clear cell, 11 papillary, 11 chromophobe) and 13 adjacent normal tissue specimens = 13 matched pairs
- HRMAS-MRS was performed on a Bruker AVANCE spectrometer operating at 600 MHz
- A MatLab-based curve fitting program developed by our laboratory was used to process the spectra to produce relative intensities for each analyzed spectral region of interest
- **Outcome:** Metabolites indicative of renal cell carcinoma
- **Statistics:** False discovery rates (FDR) were used from the response screening to account for multiple testing. Regions of interest (ROI) with FDR <0.05 were selected as potential predictors of malignancy
  - Wilcoxon rank sum test was used to compare the median MRS relative intensities for those metabolites that may differentiate between malignant and adjacent benign tissue
  - Wilcoxon signed rank test was used to compare paired RCC and adjacent benign samples

RESULTS

Table 1. Baseline characteristics and metabolomic predictors of malignancy

	RCC (N=38)	Adjacent benign parenchyma (N=13)	P-value
Age (years)	55.3 ± 11.4	50.8 ± 7.3	0.1818
Males (n, %)	27 (71.1)	8 (61.5)	0.7302
Race (n, %)	37 (97.4)	13 (100)	1.00
Median MRS relative intensities (IQR)			
4.07-4.05 (Myo-Inositol)	0.80 (0.48, 1.32)	1.84 (1.27, 2.24)	0.0026
4.02-4.00 (TBD)	1.21 (0.68, 2.07)	0.50 (0.06, 0.88)	0.0073
3.99-3.96 (Histidine, Phenylalanine, Phosphocholine, Serine)	1.26 (0.84, 1.93)	2.56 (1.19, 3.50)	0.0092
3.95-3.94 (Serine, Phosphocreatine)	0.77 (0.33, 1.24)	0.30 (0, 0.53)	0.0006
3.93-3.91 (Creatine, Glycerophosphocholine)	1.28 (0.90, 1.61)	0.69 (0.24, 1.34)	0.0071
3.61-3.59 (Myo-Inositol, Glycerophosphocholine, Phosphocholine, Valine)	0.96 (0.63, 1.24)	1.68 (1.39, 1.96)	0.0006
3.55-3.52 (Glycine)	1.92 (0.77, 3.17)	4.02 (2.87, 4.42)	0.0019
3.36-3.34 (Scylla-Inositol)	0.55 (0.35, 0.78)	1.34 (0.75, 1.54)	0.0019
3.24-3.23 (Myo-Inositol, Taurine)	5.86 (3.95, 9.46)	4.32 (2.43, 5.40)	0.0267
3.22-3.21 (Phosphocholine, Glycerophosphocholine, Histidine)	0.69 (0.22, 2.16)	4.23 (3.05, 5.53)	<0.001
3.15-3.13 (Spermine, Histidine, Phenylalanine)	0.21 (0.11, 0.35)	0.83 (0.49, 1.02)	<0.001
2.84-2.82 (TBD)	0.28 (0.18, 0.45)	0.18 (0.10, 0.23)	0.0021
2.45-2.42 (Glutamine)	0.51 (0.30, 0.74)	0.32 (0.21, 0.38)	0.0098
2.15-2.11 (TBD)	1.45 (1.15, 1.97)	1.95 (1.46, 2.50)	0.0370
1.93-1.92 (Acetoacetate)	0.31 (0.18, 0.67)	0.77 (0.54, 2.83)	0.0008
1.35-1.33 (Lactate)	8.74 (5.26,13.23)	5.2 (3.06, 8.30)	0.0150

*TBD denotes that the specific metabolites characterizing this region remain to be identified

Figure 1. Radar plot of metabolomic predictors of malignancy

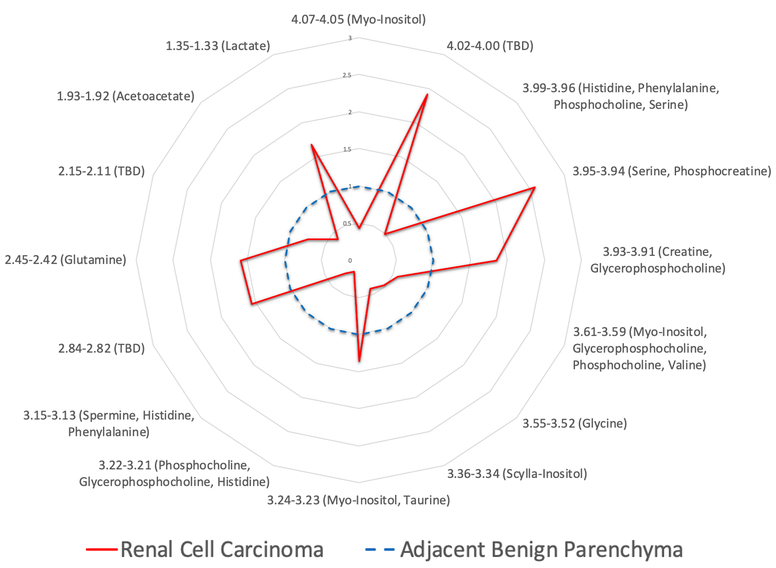

Table 2. Odds ratios for risk of malignancy for metabolites identified as potential predictors of malignancy based on FDR P-value (reference group: adjacent benign)

Region of interest	FDR P-value	Odds ratios (OR, 95% CI)	P-value for OR
4.07-4.05 (Myo-Inositol)	0.027	0.38 (0.18, 0.82)	0.013
4.02-4.00 (TBD)	0.034	3.12 (1.10, 8.84)	0.032
3.99-3.96 (Histidine, Phenylalanine, Phosphocholine, Serine)	0.013	0.34 (0.16, 0.71)	0.004
3.95-3.94 (Serine, Phosphocreatine)	0.003	29.2 (2.47. 345.24)	0.007
3.93-3.91 (Creatine, Glycerophosphocholine)	0.012	8.17 (1.77, 37.78)	0.007
3.61-3.59 (Myo-Inositol, Glycerophosphocholine, Phosphocholine, Valine)	0.005	0.13 (0.03, 0.49)	0.003
3.55-3.52 (Glycine)	0.024	0.59 (0.39, 0.90)	0.014
3.36-3.34 (Scylla-Inositol)	0.005	0.08 (0.02, 0.42)	0.003
3.24-3.23 (Myo-Inositol, Taurine)	0.030	1.35 (1.04, 1.76)	0.027
3.22-3.21 (Phosphocholine, Glycerophosphocholine, Histidine)	<0.001	0.41 (0.35, 0.67)	<0.001
3.15-3.13 (Spermine, Histidine, Phenylalanine)	<0.001	4×10^{-5} (7.42×10^{-8}, 0.02)	0.001
2.84-2.82 (TBD)	0.009	7158.67 (6.3, 8.3×10^{6})	0.013
2.45-2.42 (Glutamine)	0.017	121.5 (2.16, 6820)	0.02
2.15-2.11 (TBD)	0.035	3.96 (1.18, 13.28)	0.026
1.93-1.92 (Acetoacetate)	0.012	0.38 (0.13, 1.09)	0.072
1.35-1.33 (Lactate)	0.033	1.22 (1.03, 1.45)	0.023

CONCLUSIONS

HRMAS-MRS identified a number of metabolomic biomarkers that may be useful predictors of RCC. In particular, the metabolomic profile demonstrated that metabolites in the 3.14-3.13 ppm spectral region was present in lower levels in malignant tissue, while higher levels of metabolites in the 2.84-2.82 ppm region substantially increased the risk of RCC. These findings warrant further investigation in a larger population for validation.

DISCLOSURES

Conflicts of Interest: None.
Funding: NIH Grant #CA115746

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